CARRIER STATE IN LEPTOSPIROSIS-INFECTED ANIMALS FOLLOWING VACCINATION WITH L. POMONA BACTERIN

by

SOHAN LAL ISSAR

G. V. Sc., Bengal Veterinary College,
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INTRODUCTION

A group of infectious diseases in man, resembling what is now called Leptospirosis, have been described under different names as early as 1812 by Larrey (60), p. 5. It is during only the past half century that extensive studies on this condition have brought to light the existence of leptospirosis both in man and animals in all parts of the world. Leptospirosis was first described as a disease of man and was known as Weil's disease, caused by Leptospira icterohaemorrhagiae, and in this connection a lot of work seems to have been done in the domain of medical research in the past. Within the last decade and a half these thin-coiled, so-called spirochetes with hooked ends (belonging to the genus Leptospira) attracted the attention of the research workers. The importance of leptospirosis in animals probably did not receive sufficient recognition in the field of veterinary research until cases of enzootic jaundice in dogs were detected, and the infective agent Leptospira icterohaemorrhagiae was recovered in England in 1925 by Okell (42). In the United States, the species that usually causes the disease in dogs is L. canicola although in some cases L. icterohaemorrhagiae has also been recovered. The species causing leptospirosis in swine and cattle is Leptospira pomona, which is evidently very widely distributed in all the major livestock producing areas in the United States. The main topic will be limited to cattle in regard to L. pomona, however, the allied problems in other species of animals are interrelated, hence merit a brief mention.

Leptospiras are found in rodents which are said to transmit the infection to man and domestic animals. Leptospira icterohaemorrhagiae is harbored by wild rats, and produces Weil's disease in man and yellow jaundice disease in dogs. In addition to this, there are other animals which often act as reservoirs and play a conspicuous role in the spread of the disease to man and animals. The canicola specie causes infection in dogs called Stuttgart's disease and canicola fever in man. The pomona species is commonly found in swine and is responsible for abortions and stunted growth and is associated with swine herd's disease in man. In cattle, L. pomona produces bloody mastitis, hemoglobinuria, decreased or abnormal milk, abortions, poor bodily condition, and death. In horses, it causes periodic opthalmia. Leptospira pomona is the most important in view of the havoc it causes, and is of vital concern to the livestock industry.

Leptospirosis in this country causes an annual loss of more than $112 million (62). The losses in cattle are attributable to death, abortions, loss of weight gains, decreased milk production, etc. In addition to these pecuniary losses to the livestock industry, carrier animals remain as a source of danger to human and animal health. Cases in slaughter house workers handling infected meat (perhaps during the acute stage of leptospiraemia) have been reported. The significance to public health, therefore, cannot be minimized.

There seems to be a general belief that carrier animals shed virulent leptospira in urine, and susceptible animals acquire this malady by direct or indirect contact with the infected urine.

From the epidemiological point of view and to combat the formidable unerring forces of any infectious or contagious disease, it is highly mandatory to determine the sources of infection responsible for the dissemination of the disease. Extensive serological surveys have been made by many laboratories over the United States, and ostensibly the whole picture on diagnosis seems to be based on serological tests.

The object of this study was to determine if leptospira organisms could be isolated from serological positive animals, and to determine if vaccinated animals remained carriers of the infection.

REVIEW OF LITERATURE

A historical review of Leptospirosis reveals the existence of this disease in man under a variety of names in the past. This disease was first described in man. Weil, in 1886, as cited by Woodwart (60), p. 57, described several cases of human leptospirosis in spite of the fact that the infective agent was unknown to him.

Theiler (61) in South Africa reported the existence of thin spiral-shaped organisms in a febrile condition of cattle with definite pathological features. Stimson (57) in New Orleans was first to see the pathogenic leptospiras in sections of kidney of a patient believed to have died of yellow fever. These so-called pathogenic spirochaetes were first observed and isolated by Inada and Ido (29) in 1915 in Japan, and they accordingly named it spirochaeta icterohaemorrhagiae. This outstanding discovery cited

by Broom (60), p. 4, was confirmed in 1915 by two groups of independent workers in Germany who encountered cases among German soldiers.

Noguchi (40) was able to isolate the spirochaete from the kidneys of wild rats near the New York harbor and classified them under the new genus leptospira because of their fine and minute windings. Noguchi (41) isolated strains of leptospira from cases of "yellow-fever" which he named L. icteroides. Wadsworth, et al. (63) recorded the first case of leptospirosis which infected a laboratory worker engaged in examining wild rats in the Hudson River Valley.

In 1939 Dr. B. Walch-Sorgdrager (64), in his excellent treatise on leptospirosis, reviewed the leptospirosis infections of man and animals. He cited the species Leptospira pomona as producing Pomona fever, swamp fever, swineherd's disease in man, and leptospiral infections in pigs.

A lot of work seems to have been done in the past in Australia in connection with L. pomona which derived its origin from the town of Pomona. In Australia, leptospirosis was not diagnosed until 1934, when an outbreak occurred among cane-field workers, and was due to Leptospira Australis A.

Shortly afterwards, Clayton, et al. (10), reported isolation of Leptospira pomona from a dairy farmer near the town of Pomona.

Sipple and Atwood (60), p. 143, outlined reports of isolation of organisms in the pomona group by Tierskich in Russia in 1942; Gsell in 1946 and 1949; Collier in Java in 1948; and Savino, et al. in Argentina. Cochenour, et al. (21), in the United States

isolated L. pomona from swine urine in an explosive outbreak. The isolation of L. icterohaemorrhagiae from swine has been reported by numerous investigators.

Sippel (51) reported surveys over the world that showed an incidence as high as 59 per cent. In the United States, Boyer (5) reported that, on the basis of high agglutination titer for leptospirosis and negative titer for brucellosis, some 40 abortions two to three weeks prior to normal farrowing time were believed due to leptospirosis. A high titer was also encountered in five heifers on premises where nephritis had occurred, and the author stated that cattle probably were the source of the swine infection. Burstein, et al. (7) made use of two approaches while reporting the incidence of Leptospira pomona infection in swine in the United States. Serological surveys made of 285 serum samples collected in large packing houses gave these results: 22 per cent were found to be positive; additional studies on 39 samples of swine anti-serums, representing over 6,000 hogs, revealed that all contained leptospiral antibodies. In the other, since experimental L. pomona infection was characterized by the pigs having interstitial nephritis, and since many normal swine coming to slaughter have similar lesions, the author made use of swine slaughtered at abattoirs. The results showed that a significant number of swine with these lesions also had evidence of leptospirosis. As an incidental finding, it was observed that 50 per cent of the kidneys from the 114 swine studied had histopathological lesions. Although these studies suggested that many of the

renal lesions present in swine are produced by leptospirosis, they also indicated that there are other causes.

It can now be inferred that leptospiral infection in swine is world-wide in distribution, and the main species involved are L. pomona and L. icterohaemorrhagiae.

In the past, leptospirosis in horses has also been reported in Europe as well as in the United States. A chronic disease condition known as Periodic opthalmia or moon blindness is now recognized as associated with Leptospira.

In Switzerland, Heusseur (26) described a study of 276 cases of periodic opthalmia reported by Swiss practioners in questionnaire replies indicating that the disease was associated with wet, swampy regions. Since opthalmia had been reported in camels in military units in which the personnel had leptospiral "field fever," the possibility of leptospira being associated with equine periodic opthalmia was investigated. The author made agglutination tests with various leptospiras as antigens and sera of 291 healthy horses and 263 periodic opthalmia cases. Of the latter group, 73 of the acute cases showed a serum titer of 1:400 or higher: 45 against grippo-typhosa, 17 against pomona, and 11 against Australis. Transmission experiments proved inconclusive. The author considered that the epizootiology and the agglutination test results pointed strongly toward leptospiras being associated with the etiology of periodic opthalmia.

Schofield of Ontario (49) reported a nonspecific hemoglobinuria from which he isolated Clostridium perfringens. The author postulated that toxic substances due to bacterial action were

formed in the intestine and upon being absorbed, caused the destruction of the red blood cells.

Smith (53) reported on Idiopathic Hemoglobinuria in cattle, and the occurrence of this disease in different parts of the United States. He stated that it had no relation to age, season, sex, care, or feed. It had a morbidity of from five to 35 per cent and a mortality of 100 per cent in untreated animals. The writer described the symptoms and post mortem lesions as observed in serious cases. He also mentioned that this condition differed from classic "red water," a disease caused by Clostridium hemolyticum, or parturient hemoglobinuria of dairy cows due to aphosphorosis, but no specific causative agent was established.

Cooper (11) reported the etiology of Idiopathic hemoglobinuria in the midwestern beef calves was due to leptospirosis. In California, the isolation of Clostridium hemolyticum in new areas and incrimination of Clostridium perfringens, type A, was also reported. In the writer's opinion it would seem safe to suggest that in the past, the term Idiopathic hemoglobinuria may have been applied to disease conditions caused by several different agents, some of which are probably not yet recognized. He also mentioned differential diagnosis of all cases of hemoglobinuria due to Clostridium hemolyticum, Leptospirosis, and Clostridium perfringens, type A.

Lukes, et al. (33) presented a study of hypertrophic liver cirrhosis in horses observed in Germany and Czechoslovakia. The authors described the sequence of the changes of the liver from the initial serous infiltration to the final chronic cirrhotic state.

As a causative agent, a leptospira was incriminated.

In the United States, Roberts, et al. (46) reported an outbreak of leptospirosis in horses. The author described six of 16 horses which developed a septicemic disease with pyrexia and icterus; one mare aborted and one developed periodic ophthalmia. Blood from two acute cases produced fever and typical leptospirosis lesions on passage through guinea pigs; leptospira organisms were observed in guinea pig peritoneal fluid, and L. pomona was recovered.

The incidence of bovine leptospirosis has been reported in the past from different parts of the world as well as in the United States.

In Russia, Michin and Azino (36) reported for the first time the occurrence of leptospirosis in cattle.

In Australia, Sutherland and Simmons (58) reported three outbreaks of leptospirosis in calves. They stated that symptoms and lesions were fever, acute anemia, hemoglobinuria, and icterus. Guinea pigs inoculated with tissue from affected calves showed leptospira in the liver but no symptoms. Calves inoculated with tissues from affected animals or guinea pigs showed symptoms of the natural disease.

Peterson (43) reported that L. pomona appeared to be the main strain involved in leptospirosis in calves in western Australia. Adult cattle and pigs were also affected, the leptospiras being excreted in the urine.

Simmons, et al. (50) reported isolation of Leptospira pomona from calves in two outbreaks of bovine icterohemoglobinuria.

In England, Field and Seller (17) reported for the first time Leptospira infection in calves. They stated the results in this case were due to Leptospira icterohaemorrhagiae. Although the patient showed a very severe icterus, experimentally infected calves did not necessarily develop jaundice. Agglutinins were found in the sera of an adult cow, indicating previous infection.

In Algeria, Donatien, et al. (14) described the histopathology of the lesions and the morphological characteristics of the leptospira from the liver and the kidney of one case. They stated that leptospira resembles the Leptospira icterohemoglobinuriae Vitulorum of Michin and Aginov.

In New Zealand, Hartley (25) reported the occurrence of leptospirosis in cattle and sheep. Diagnosis was made on the basis of lesions containing leptospiras, similar pathologically to bovine leptospirosis. He transmitted viable leptospiras to guinea pigs.

In Canada, Smith and Perry (52) reported bovine leptospirosis. The organisms were demonstrated in Levaditti-stained sections of kidney and liver. The authors also stated that other earlier cases in which the organisms were not demonstrated may have been leptospirosis. Urine was cited as the major factor in the spread of the infection.

In the United States, the existence of bovine leptospirosis was first reported by Jungherr (31). The author found the leptospiras in stained tissue sections of diseased cattle after necropsy.

Mathews (34) reported the presence of leptospira in the membranes of aborted fetuses. The author found leptospira-like structures in silver-stained preparations of the fetal membranes.

Baker and Little (1), for the first time, successfully isolated leptospira as the causative agent of bovine leptospirosis in a natural outbreak of the disease in New Jersey. The authors reported that from abnormal milk of cows, an agent was transmitted to guinea pigs, rabbits, mice, and embryonated eggs, which caused febrile reaction in guinea pigs and rabbits. In early passages, embryonated eggs were unaffected but later, death of embryos occurred seven days post inoculation. They demonstrated experimental infection in young calves by subcutaneous and intranasal inoculation with blood from infected guinea pigs or chorioallantoic fluid from infected eggs. In calves, it produced fever and albuminuria; in lactating cows, the infection resembled that seen in animals with natural disease. They stated further that pen contact of normal cows and calves with infected calves resulted in non-apparent infection. The agent was shown to cause interstitial nephritis in addition to causing altered milk. The agent was recovered from blood and milk during the febrile stage and also was demonstrated in urine long afterwards. The authors also found the antibodies in the sera of experimental animals and of cows recovered from the natural disease.

Slatter, et al. (54) reported diagnosis of leptospirosis in young calves by dark field examination and check embryo cultures; previously some of these cases were diagnosed as general debility due to poor nutrition and to calfhood infections.

Little and Baker (32) described severe and mild forms of outbreaks in New Jersey that occurred in the period, May to November. The symptoms, as stated by the authors, were hemoglobinuria;

bloody milk and a soft, limp udder; pregnant animals most likely abort; weakness; anemia; and nephritis. In the mild form, similar symptoms but less severe, lasted two to four days; with depression, anorexia, dyspnea, abortion, and drop in milk secretion, with a temperature of 102° to 105° F., as compared to 103° to 107° F. in the severe form. They stated that spirochetes can be recovered from the blood and milk, and occasionally the urine during the febrile period. Subcutaneous or intranasal inoculation of such products may reproduce the condition. They further recommended that material must be promptly used, the blood should be from febrile cows, the milk should be bloody, thick or viscid, and the urine abnormal in appearance. For isolation, infected guinea pig blood or allantoic fluid from infected eggs should be inoculated into Schueffner's medium. Levaditi's stained liver and kidney sections also demonstrate the spirochete. Agglutination or lysis of suspensions of organisms appeared when the serum of affected animals was employed two to four weeks after disease. Titers of 1:20 were considered suspicious; of 1:200 or above, positive. The authors were of the opinion that insect vectors or carrier animals may be responsible for transmission. The organism was recovered from the urine 53 days after apparent recovery, and observed in kidneys eight weeks after infection was recognized. The authors also stated that intranasal inoculation of urine from infected cases transmitted the infection to normal animals. The authors stressed that carrier animals be recognized by determining those with antibodies, and whether the organism was present in the urine.

Cochenour, et al. (20) first reported the identification of Leptospira pomona as the predominant species involved in bovine leptospirosis in the United States.

Reinhard (44) reported the clinical pathology in the study of experimental leptospirosis of calves. The author observed that the calves subjected to inoculation with leptospiras, developed fever beginning on the fourth day and lasting for four days. Leptospiremia occurred in a few animals and disappeared when the fever terminated. The author stated that albuminuria was associated with leptospiruria after the fever. Hemolytic anemia, hemoglobinuria, with an increase of red cell fragility occurred. Kidneys revealed focal interstitial nephritis and liver focal necrosis in a fatal case.

Schaeffer (48) reported a water-borne outbreak of a disease involving about 50 persons who became ill after swimming. He stated that 26 members of this group kept under observation, gave proof that infection was attributable to L. pomona.

Cardy and Haunz (8) reported an outbreak of Leptospira canicola infection in North Dakota involving nine members of one family.

An editorial (15) published in 1952, York's Survey of Cattle in New York, revealed that a large proportion (12.8 per cent) of the dairy cattle were infected with leptospirosis.

Spink (55) reported human leptospirosis due to L. pomona in a group of individuals engaged in the meat packing industry.

Cordy and Jasper (12) reported the pathology of an acute hemolytic anemia of cattle associated with leptospira. The authors described 11 outbreaks of cattle in California, and the finding of leptospira in stained sections of 16 animals; usually animals under

eight months of age were involved with a history of hemoglobinuria and sudden death. They observed icterus, small hemorrhages in the peritoneum, collection of blood pigments, stromal leucocytic infiltration in the renal tubules, epicardial petechia, and centrolobular hepatic necrosis associated with dilatation of the sinusoides and adjacent degenerative changes; leptospira in renal tubular lumina and also in the hepatic sinusoids.

Bohl and Ferguson (2) reported that serological evidence showed that infection with Leptospira pomona is widespread among cattle, swine, and horses in Ohio. The authors stated that no antibodies for Leptospira grippotyphosa, L. bovis, L. autumnalis, or L. bataviae could be found in cattle, horses, or swine in Ohio. They isolated L. pomona from the kidney of a healthy pig which was serologically positive and also confirmed as L. pomona by serological identification. The authors discussed the possibility of transmission of L. pomona from cattle to swine and vice-versa; also the role of serologically positive swine with no manifestation of illness.

Reinhard (45) reported the presence of seven known leptospira species in the United States. The author stated that the major species in bovine leptospirosis is L. pomona in the United States. Leptospiral infections of man by L. pomona, swineherd's fever, and by L. autumnalis, Fort Bragg fever, have been found. Porcine and equine leptospirosis caused by L. pomona have been discovered more recently and are as widespread as bovine leptospirosis. The writer stressed the importance of the huge animal reservoirs of human pathogens as a serious public health problem.

The author also discussed the developments of the past decade, symptomatology, clinical pathology, serology, immunology, epidemiology, and control.

Yager, et al. (65) reported the first occurrence of Leptospira ballum in rural house mice and in an oppossum. The authors stated that in a search for wild life carriers of L. pomona, various species of mammals were trapped in an endemic area of bovine leptospirosis. Leptospiral culture isolates were obtained from 15 of 37 housemice and one of two oppossums. All isolates belonged to the sero-type L. ballum. The authors concluded that the failure to detect L. pomona in the rodent and other wild life population in an endemic area is evidence that the natural hosts of L. pomona are to be found primarily in the livestock population.

Gillespie, et al. (18) reported the isolation of leptospira from cattle in Washington. The authors, in a preliminary report, stated that two to three milliliters of centrifuged bovine urine sediment was inoculated into young guinea pigs. When their temperatures reached 104° F., blood was withdrawn for inoculation into a two-day-old guinea pig, and for culture and serologic studies. They recovered the leptospira by this technique, and serological proof also was secured for the presence of the disease in the State of Washington.

Stevenson, et al. (56) reported the isolation of Leptospira mitis as well as L. pomona in Victoria. The authors described a serological survey of 422 Milbourne metropolitan abattoir workers that gave evidence of infections with L. pomona or L. mitis in 7.6 per cent of the men. The authors investigated a small outbreak

of leptospirosis in a city abattoir. In Melbourne, three cases and a possible fourth of meningitis due to L. pomona were diagnosed.

Hoag and Wilson (27) reported isolation of L. pomona from the aqueous humor of the anterior chamber of the eye of a calf inoculated intramuscularly with first passage of L. pomona. The authors further stated that this calf developed transient conjunctivitis and uveitis. The other calves subsequently inoculated with calf passage L. pomona did not show eye lesions.

Hoag and Wilson (28) reported the occurrence of a bovine leptospiral meningitis in a two-year-old steer exhibiting the symptoms of fever, stiffness, conjunctivitis, excessive salivation, etc. The authors further stated that serum of this animal was positive at five days, 15 days, and 30 days on agglutination lysis test and that the animal recovered in about a week, after losing 50 pounds of weight. When slaughtered after a month, the animal appeared normal.

Covaleda and Pumarola (13) of Spain, reported that serum agglutination tests revealed that of 122 pigs slaughtered in Barcelona, 19.6 per cent of apparently healthy animals were or had been carriers of Leptospira pomona. Another lot of 92 pigs showed the presence of L. pomona in 13 per cent; of L. mitis in 17.3 per cent; and L. icterohaemorrhagiae in 3 per cent. The authors were of the opinion that hogs, at least in Spain, may act as reservoir hosts for leptospiral organisms and may therefore be a definite source of infection for man.

Newman (39) stated in his preliminary report that of 241 blood samples submitted for testing, 52 or 21.6 per cent were positive and 8 or 3.3 per cent were suspicious on the agglutination lysis test with L. pomona antigen. Infection was present in 21 herds when 190 random samples submitted for routine brucellosis examination were tested; 6 or 3.1 per cent were positive and 4 or 2.1 per cent were suspicious for leptospirosis. The author concluded that infection is present in 14 Michigan counties.

Stoenner (59) reported on management of herds affected by bovine leptospirosis. The author stated that epizootics of bovine leptospirosis due to L. pomona were frequently associated with urine contaminated water. In the writer's opinion, the disease is usually a self-limiting herd problem, depending upon the size of the herd, environment, and introduction of susceptible cattle. The author described variable infection rate, mortality and morbidity higher in rapidly-spreading epizootics, loss from abortions of 20 to 40 per cent, sterility, anemia, drop in milk yield, and weight loss. In his opinion, serological reactions indicated significant herd infection before the infection was recognized. The author recommended that cattle with positive reactions and not shedding the organisms should be immune and hence, retained. Further, he stressed husbandry methods to avoid contamination of feed and water, and isolation of infected animals from non-infected animals, and the feeding of pasteurized milk to calves. He recommended vaccination of the entire herd in an early outbreak, although this is controversial. Calves nursing serologically positive cows receive passive immunity. In the author's

opinion, the use of antibiotics also was controversial.

Bohl, et al. (3) described their observations in a herd of 29 pregnant sows; only seven normal litters of pigs were farrowed during the season. Abortions or the birth of a high proportion of dead or weak pigs were detected in 16 sows who did not manifest any sickness. Of 19 sows tested, all proved serologically positive to L. pomona and had a negative titre for brucellosis. The authors observed great numbers of leptospiras from the kidney and liver suspensions made from a weak pig which had been born only six hours previously. Leptospira pomona was isolated from the blood and kidney of this pig and was considered the cause of abortions. While no contact was had with cattle, 83 per cent of 41 cattle on this farm were serologically positive and four abortions had been observed. The authors suggested that in infection, leptospiremia occurs and the fetus is readily susceptible to leptospiras.

Hall and Bryans (23) reported a case of leptospirosis in a horse due to L. pomona. The writers based their diagnosis on a progressive rise in serum antibody titer against L. pomona. They stated that guinea pig inoculations and blood cultures were unsatisfactory, and the source of infection could not be determined.

Bryans (6) demonstrated the presence of leptospiral agglutinins in the serum and aqueous humor of horses affected with periodic ophthalmia. Two cases of periodic opthalmia were observed in horses known to have been naturally infected with Leptospira pomona. In one case, ophthalmia occurred a year after infection and in the other horse, a two-year period elapsed.

The author was unsuccessful in his attempt to recover the leptospira in culture from six cases of periodic ophthalmia. Thirty per cent of 512 serums from horses had agglutinins for L. icterohemorrhagiae. In the author's opinion, agglutinins may persist for more than two years and serological diagnosis must be based on a rising agglutinin titer. Studies on experimental infection with L. pomona in six horses showed hemolytic anemia with grossly visible icterus. Leptospira pomona was recovered during the early febrile stage. The writer stated that an attempt to isolate leptospira from the urine of experimentally-infected animals was unsuccessful.

Hadlow and Stoenner (22) described the histopathology of organs from 15 Hereford cows which had aborted several months following spontaneous infection with L. pomona. They stated that the primary finding was an extensive chronic focal interstitial nephritis. The inflammatory cells consisted of small and large lymphocytes and less frequently, neutrophils and esinophils. Renal tubular cell proliferation was a common finding, where it was associated with a defective or dissolved basement membrane. Proliferating tubular cells formed bizarre syncytia and giant cells. A few leptospiras were seen in the kidney sections from six of the 15 cows. Hemosiderosis was the most frequent finding in the spleen. Hepatic lesions revealed only limited portal and interlobular mononuclear cell infiltration. No significant changes could be found in the lungs and uteri.

York, et al. (66) investigated the use of vaccine in the control of leptospira in cattle and swine. They stated that eight

cattle known to have had a natural attack with Leptospira pomona proved refractory to experimental infection. Field experience with a 360-head herd indicated that recovered animals did not become reinfected. They vaccinated 21 calves two to five months of age with five cubic centimeters of vaccine. When challenged with virulent L. pomona, none of them developed infection. Swine similarly vaccinated, showed serum titers of 1:40-320. Cattle from different infected herds were also vaccinated. Two hundred seventy-seven of these had 37 with agglutination lysis titers of 1:200 at vaccination; after six weeks, there was an increase of 2.1 per cent of such titers. In 152 unvaccinated controls, there were 15 with titers and six weeks later, 28 per cent new reactions appeared.

Morse and McNutt (37) investigated the course of L. pomona infection in pregnant heifers. They inoculated seven pregnant heifers with L. pomona subcutaneously. One developed serious illness, one aborted, and five remained asymptomatic. According to them, leptospiremia lasted for one to five days in five of the animals while seven developed a transitory leptospiruria as evidenced by isolation of the agent from urine or kidneys. Leptospira pomona was recovered from the udder, cotyledons, liver, spleen, lungs, and kidneys of one acutely ill animal killed 10 days after the onset of infection. Leptospiras were not isolated from the tissues of any of the fetuses from these heifers. Antibodies in serum were detectable within 10 to 16 days following exposure to L. pomona.

Morter and Morse (38) reported on the role of six calves subjected to experimental infection with L. pomona in spreading the disease to other animals. Of this lot, three Herefords developed hemoglobinuria; one died on the eighth day post-inoculation and another on the 25th day. All six developed leptospiremia and four shed leptospira in their urine. This infection was spread, by contact, to three of four pregnant heifers, to all of four pigs, to one of two goats, but to none of four sheep. Clinical symptoms were manifested six to 24 days after exposure. They further discussed the course of leptospirosis in calves, heifers, pigs, and a goat as well as the serological results following infection.

Borg and Fennestad of Denmark (4) reported isolation of Leptospira pomona from a field rodent. They stated that L. pomona previously not known to exist there, was isolated from the kidneys of three of 14 specimens of the striped field mouse, Apodemus agrarius, a rodent found in Denmark. On the island of Lolland and Falster, L. pomona antibodies were found in 13 of 200 sera of cattle and in five of 153 sera of swine in the same area, but not in serum from 1200 cattle or 345 swine from other areas. The authors said that in Denmark, the striped mouse is believed to be the principal carrier of L. pomona. They explained that the higher rate of infection in cattle than in swine is due to management which favors contact between field rodents and cattle.

Chalquest (9) reported the feeding of diluted urine from a heifer with leptospiruria produced a positive agglutination lysis test for L. pomona in four of six White Leghorn cockerels; two of

three Hungarian partridges; one of eight Pekin ducks. The author was unsuccessful in his attempt to demonstrate the transmission of leptospiras in the excreta of the birds positive to the agglutination lysis test for L. pomona by inoculating diluted fecal samples intraperitoneally into two-day-old chicks.

Ferguson, et al. (16) reported isolation of a strain of Leptospira pomona from the urine of a cow during a natural outbreak of leptospirosis; it also was used to expose 12 experimental cattle. All of these animals became infected, and a yearling bull developed acute leptospirosis and died on the seventh day. They demonstrated leptospiras in the blood of 11 of the 12 cattle one or more days of the first week. The cow which was negative in blood culture did not shed leptospira in the urine at a later time. Leptospira pomona became localized in the kidneys and was shed in the urine of eight of the 12 cattle between 13 and 102 days following infections. Three of nine pregnant cows aborted fetuses on days 19, 20, and 47. No leptospira were demonstrated in the fetuses by direct cultural examination or by animal inoculation of various tissues. The authors suggested that a toxic substance is released in the tissues from leptospiras when they are lysed by antibodies. They were of the opinion that such a toxin may cause the destruction of erythrocytes and other tissues of the infected cow and presumably may pass the placenta and cause the death of the fetus. This would explain the inability to demonstrate L. pomona in the aborted bovine fetus. The histopathological lesions were found to be similar to those in acute fatal leptospirosis and changes observed in the kidneys of chronic

carriers in the course of natural attack.

Hamdy and Ferguson (24) reported in their investigation that L. pomona in early passages readily infected hamsters. Mortality was low, but when the hamsters were killed 84 days later, the kidneys were grossly involved, and leptospiras were demonstrated by dark field examination. According to the authors, the strain increased in virulence as the passages progressed. At the 16th passage, the hamsters died within four days; by the 20th passage, all died on the third day. At the 26th passage, the virulence declined and the hamsters died after six days.

Gillespie, et al. (19) reported the isolation of five strains of Leptospira pomona from surface waters from different streams, natural drainage basins, and from ponds on different ranches. They recovered Leptospira pomona in every instance from cattle shedding the leptospira in urine.

Menges, et al. (35) reported on the diagnosis of leptospirosis from urine specimens by direct culture. The authors stated the bladder-typing technique on living animals which they satisfactorily used on dogs, and were of the opinion that it can be used on pet animals, sheep, swine, goats, and young calves. They also recommended that technique at necropsy may be used for surveys at packing plants and wild animal surveys. They were able to isolate leptospira from urine by this technique from five rats, 26 experimentally-infected guinea pigs, and from seven dogs. These dogs were known L. canicola carriers or experimentally-infected dogs.

MATERIAL AND METHODS

The material used in this series of experiments was obtained from two sources: (a) the Kansas State College Dairy herd and (b) field cases suspected for leptospirosis that were brought to the Veterinary Clinic. The field cases were used to determine if isolations could be made by the methods employed. Blood samples were drawn from the cattle in the dairy herd, and the plate agglutination test was conducted on the serum. This test was conducted in June, 1957, and 66 per cent of the serum samples reacted positively to the agglutination screening tests. In the latter part of August, 1957, there occurred a storm of abortions (17 animals) in the dairy cattle herd. After the abortions had occurred, the cattle in this lot were again subjected to a plate agglutination test. The animals with a high titer were selected for this experiment. The cattle in the dairy herd were vaccinated after the abortions with Leptospira pomona bacterin. Actual collection of material for study was undertaken after vaccination in early October, 1957. Blood samples were drawn from cattle under experiment during the months of November and early December, 1957, and an agglutination test was conducted to determine if the titers had changed since the storm of abortions in August.

Young hamsters weighing 75 to 100 grams and young guinea pigs weighing 200 grams to 250 grams were identified with ear tag numbers and used as test animals. These animals were divided into two groups: (1) in the first group, the hamsters or guinea pigs were inoculated by the intraperitoneal route with 1 to 5 cubic

centimeters of whole blood at the time the blood was withdrawn from the cattle; (2) in the second group, the hamsters or guinea pigs were inoculated by similar route with 0.5 to 1 cubic centimeter of centrifuged urine sediment. The temperature of hamsters and guinea pigs under experiment was recorded before the inoculation of material and for eight days post-inoculation. The guinea pigs inoculated with material from the dairy herd cattle were regularly subjected to serial passages on the fourth or fifth day irrespective of any thermal reaction. Before this, transfer passages were conducted only in cases where febrile reaction was observed in animals under experiment.

For cultural studies, Modified Stuart's Medium was used which contains the following ingredients:

Sodium chloride	1.93 grams
Asparagine	0.13 grams
Ammonium chloride	0.27 grams
Magnesium chloride (hyd)	0.19 grams
Sodium phosphate dibasic (anhyd)	0.67 grams
Potassium phosphate monobasic	0.087 grams
Thiamine hydrochloride	2.0 mg.
Distilled water	1.0 liter

The ingredients were thoroughly mixed and the pH was adjusted to 7.6. This media (9 milliliters was placed in tubes and autoclaved at 121° C. for 20 minutes. The pH was again tested after autoclaving. Sterile rabbit serum (10 per cent) was added to each tube before inoculation. The sterility of the rabbit serum was

tested in nutrient broth.

Material (in most cases) was collected on five occasions from each animal under experiment in the dairy herd.

Blood

Whole blood was withdrawn, under aseptic precautions, from the jugular vein in cattle and inoculated (1 to 2 cubic centimeters) directly into each of two tubes of Stuart's Medium. Blood from each cow was also citrated by adding 5 cubic centimeters of blood to a tube containing 1 cubic centimeter of 3 per cent solution of sodium citrate previously dried and sterilized in a hot air oven. Citrated blood (0.1 to 0.2 cubic centimeters) was inoculated into each of two tubes of Stuart's Medium.

Urine

Urine was collected from each cow by means of a sterile catheter. The urine was centrifuged in four sterile 50 milliliter tubes for 30 minutes at 2,000 revolutions per minute. The supernatant was removed, and the urine sediment from the four tubes was pooled. This urine sediment, 0.2 to 0.3 cubic centimeter, was inoculated into each of two tubes containing Stuart's media.

The cultures made from whole blood, citrated blood, and urine sediment were incubated at room temperature and examined by dark field microscopy on the fifth day and then at weekly intervals for four weeks, then monthly for two months for the presence of Leptospira.

Microscopic examinations of each sample of centrifuged urine sediment were made under dark field for Leptospira. Smears of urine sediment were also made and stained with Giemsa and examined microscopically for Leptospira. These smears were stained from 4 to 12 hours in Giemsa solution.

Pathological Studies

At necropsy, tissues were preserved in 10 per cent buffered formalin from cattle that were made available for histopathological examination. Tissues of hamsters and guinea pigs which died or were destroyed were also sectioned for pathological studies. Fresh tissue suspensions of kidney were examined under dark field microscopy when animals were necropsied. The sections made from the tissues were stained by hematoxylin-eosin, and silver impregnation methods of Levaditi's and Warthin's.

PROCEDURE

During the course of this study, 14 cases from the college dairy herd and six cases from the field were examined.

Case No. I

A steer from near Alta Vista, Kansas was submitted to the Pathology Department. This animal was exhibiting signs of hemoglobinuria, cyanosis, and increased respiration, indicating an anemia. The animal's temperature was 104° F. Urine was collected and inoculated into two hamsters (Nos. 1 and 2). Cultural examinations were made of the urine sediment in Stuart's media. A direct microscopic examination was made of the urine sediment.

Case No. II

A urine sample showing hemoglobinuria was submitted to the Pathology Department. The history revealed that abortions had occurred in this herd and the owner had suspected leptospirosis. Two hamsters (Nos. 3 and 4) were inoculated intraperitoneally with 1 and 2 cubic centimeters of the urine sediment. A serial transfer of blood was made on the seventh day from these two hamsters into two other hamsters (Nos. 5 and 6). Cultural and microscopic examinations also were made on this specimen as in Case No. I.

Case No. III

An adult cow (Oak Hill, Kansas) was presented for treatment to the Veterinary Clinic. The animal died before treatment could be administered. The animal was showing hemoglobinuria and bloody mastitis. Two hamsters (Nos. 7 and 8) were inoculated with 0.2 cubic centimeters of urine and milk sediment, respectively. Hamster No. 7 died on the sixth day and hamster No. 8 died on the 15th day. Tissues were saved for pathological examination. Cultures were made in Stuart's media from blood, urine, and milk. Microscopic examinations of urine, milk, and bovine tissues were also made.

Case No. IV

A calf suffering from hemoglobinuria was submitted to the Pathology Department for necropsy. The animal was suspected as being infected with leptospirosis. A post-mortem was conducted,

and tissues were saved for pathological studies. One guinea pig (No. 16) was inoculated with 0.5 cubic centimeter of urine sediment and guinea pig No. 17 was inoculated intraperitoneally with 0.3 cubic centimeter of bovine tissue emulsion (liver and kidney). Cultures were made from urine sediment into Stuart's media. Dark field examinations were made of urine sediment and bovine tissues immediately after necropsy.

Case No. V

A calf (Westmoreland, Kansas) was submitted to the Pathology Department for necropsy during December, 1957. Leptospirosis was suspected in this calf from its history. The client lost four calves at his farm. The remainder of the herd was vaccinated for leptospirosis. One calf on the farm was found to have a temperature of 104.6° F. Ten cubic centimeters of blood were drawn from this calf and inoculated immediately into two guinea pigs (Nos. 496 and 486). Each pig received 5 cubic centimeters of blood intraperitoneally. A serial transfer of blood was made on the fifth day from these two guinea pigs into other guinea pigs (Nos. 421 and 1042). Routine cultures from heart blood were made from all four pigs.

Case No. VI

A calf was submitted to the clinic from Alma, Kansas for necropsy. This calf was dead upon arrival, but the mucous membranes were anemic, and the urine was a port wine color. Kidney

and liver tissues were inoculated into guinea pigs (Nos. 177 and 178).

Case No. VII

Urine samples were collected from cow No. 317C, once during the month of October, twice in November, and twice in December, 1957. Blood samples were collected only in October. Guinea pigs (Nos. 19, 20, 23, 436, and 472) were injected with whole blood in progressive dilutions (1 to 5 cubic centimeters). Guinea pig No. 21 was injected with 2 cubic centimeters of citrated blood. Guinea pigs Nos. 22, 24, 479, and 466 were inoculated intraperitoneally with 0.5 to 1 cubic centimeter of urine sediment. Guinea pigs Nos. 22 and 24 showed an abnormal rise in temperature, 105° F. and 103.8° F., on the third and fourth day, respectively. Two-tenths of a cubic centimeter of blood was drawn by cardiac puncture from each of these animals, and cultures were made in Stuart's media. A serial transfer of blood was made from guinea pig No. 24 into guinea pig No. 25. On the sixth day, guinea pig No. 25 showed a rise in temperature to 104° F., and heart blood cultures were made in Stuart's media. Routine serial transfer of blood from guinea pigs Nos. 436, 479, 472, and 466 was made into other guinea pigs, Nos. 420, 424, 473, and 474 simultaneously, and cultures were also made from each of these animals. Samples of urine sediment, whole and citrated blood were subjected to cultural and microscopic examinations.

Case No. VIII

Samples of urine were obtained from Cow No. 455B once during the month of October, twice in November, and twice in December, 1957. Blood was collected only during October. Guinea pigs Nos. 26, 31, 33, 470, and 425 were inoculated intraperitoneally with 1 to 5 cubic centimeters of whole blood. Hamster No. 27 and guinea pig No. 28 were inoculated intraperitoneally with 1 cubic centimeter of citrated blood. Guinea pigs Nos. 29, 32, 34, 453, and 402 and hamster No. 30 were inoculated with 0.5 and 1 cubic centimeter of urine sediment intraperitoneally. A serial transfer of blood was made from guinea pigs Nos. 453 and 402 into guinea pigs Nos. 102 and 403, and simultaneously cultures from blood were made from each of these animals. Cultural and microscopic examinations were made on urine sediment immediately after collection.

Case No. IX

Samples of blood and urine from cow No. 156B were obtained once during the month of October, once in November, and three times in December, 1957. Guinea pigs Nos. 35, 37, 418, 39, and 409 were inoculated intraperitoneally with 1 to 5 cubic centimeters of whole blood. A serial transfer of blood was made from guinea pig No. 418 into guinea pig No. 200, and cultures of the blood were also made into Stuart's media. Guinea pigs Nos. 36, 38, 455, 40, and 410 were inoculated intraperitoneally with 0.5 to 1 cubic centimeter of urine sediment. Serial transfer of blood

was made from guinea pigs Nos. 455, 40, and 410 into guinea pigs Nos. 483, 41, and 42. Direct cultures from whole and citrated blood were made only in October. Cultural and microscopic examinations were made on all urine samples soon after collection.

Case No. X

Samples of blood and urine were obtained from cow No. 20B twice during the month of November and three times in December, 1957. Guinea pigs Nos. 43, 45, 433, 435, and 440 were inoculated with 1 to 5 cubic centimeters of whole blood. A serial transfer of heart blood was made from guinea pigs Nos. 433, 435, and 440 to guinea pigs Nos. 301, 117, and 170. Cultures were also made of the blood into Stuart's media. Guinea pigs Nos. 44, 46, 434, 437, and 441 were inoculated with 0.5 to 1 cubic centimeter of urine sediment. Serial transfer of blood (one cubic centimeter) was made from guinea pigs Nos. 434, 437, and 441 into guinea pigs Nos. 215Z, 49, and 522. Cultures were also made from the heart blood into Stuart's media. Microscopic and cultural examinations were made on urine sediment from cow No. 20B.

Case No. XI

Samples of blood and urine were collected from cow No. 326B twice in November and three times in December. Guinea pigs Nos. 525, 528, 203, 220, and 235 were inoculated with 1 to 5 cubic centimeters of whole blood. Serial transfer of heart blood was made from guinea pigs Nos. 203 and 220 into guinea pigs Nos. 204 and 224, respectively. The blood was cultured in Stuart's media.

Similarly, guinea pigs Nos. 526, 529, 205, 222, and 237 were inoculated intraperitoneally with 0.5 to 1 cubic centimeter of urine sediment. On the fifth day, a serial blood transfer was made from No. 526 into guinea pig No. 527, and a blood culture was also made from this animal. The remainder of the urine-inoculated guinea pigs Nos. 205, 222, and 237 were subjected to serial heart blood transfer into other pigs Nos. 109, 232, and 238, respectively. Direct blood cultures were not attempted in this case. Routine microscopic and cultural examinations were made of urine sediment.

Case No. XII

Samples of blood and urine were collected from cow No. 102B once during the month of November and four times in December, 1957. Guinea pigs Nos. 50, 429, 1020C, 80, and 110 were inoculated with 1 to 5 cubic centimeters of whole blood. Serial transfer of heart blood (1 cubic centimeter) was made from pig No. 1020C to guinea pig No. 1021C. Guinea pigs Nos. 51, 909, 725, 81, and 101 were inoculated with 0.5 to 1 cubic centimeter of urine sediment. Blood transfers were made from the last four guinea pigs above into guinea pigs Nos. 911, 735, 91, and 102, respectively. Cultural and microscopic examinations were also made on urine sediment.

Case No. XIII

A blood sample was obtained from cow No. 159B in November, and urine was obtained once in November and three times in

December, 1957. One guinea pig (No. 170Z) was inoculated with 2.5 cubic centimeters of whole blood. Guinea pigs Nos. 300, 177Z, 665, and 249 were inoculated intraperitoneally with 0.5 to 1 cubic centimeter of urine sediment. A serial blood transfer was made from guinea pigs Nos. 1772, 665, and 249 into guinea pigs Nos. 810, 666, and 250. Heart blood cultures were also made with each serial transfer. Cultural and Dark field examinations were conducted on the urine specimens.

Case No. XIV

Blood samples were collected from cow No. 267B once during November and twice in December, while urine was collected once during November and four times in December, 1957. Guinea pigs Nos. 779, 482C, and 11Z were inoculated intraperitoneally with 1 to 3 cubic centimeters of whole blood. Guinea pigs Nos. 222, 500, 313Z, 133C, and 175 were inoculated with 0.5 to 1 cubic centimeter of urine sediment. A serial transfer of heart blood (1 cubic centimeter) was made from guinea pig No. 482C to guinea pig No. 756. Cultures from heart blood into Stuart's media were also made from each of these pigs. A serial transfer of heart blood was made from pigs Nos. 500, 313Z, 133C, and 175 into guinea pigs Nos. 501, 315Z, 137C, and 176. Blood cultures were also made from each of the four guinea pigs. Cultural and microscopic examinations were made of the urine sediment.

Case No. XV

Samples of blood were collected from cow No. 205B once in November and once in December, and urine samples were collected once in November and four times in December, 1957. Guinea pigs Nos. 1219 and 497E were inoculated with 1 and 2 cubic centimeters of whole blood. A serial transfer of blood from guinea pig No. 497E into guinea pig No. 599E was made four days post-inoculation, and cultures from heart blood were also made into Stuart's media. Guinea pig No. 1220 was inoculated with 0.5 cubic centimeter of urine sediment. On the seventh day, this guinea pig showed a temperature rise to 103.6° F. Cultures were made from the heart blood of this pig and at the same time, the blood was used in a serial transfer into guinea pig No. 1221. Blood cultures from thir pig were also made in Stuart's media. Guinea pigs Nos. 498E, 774, 667, and 569 were inoculated with 0.5 to 1 cubic centimeter of urine sediment, and a serial transfer of blood was made from each of these pigs to guinea pigs Nos. 501, 775, 668, and 679, respectively. Cultures of blood were made from the guinea pigs inoculated with urine and subsequent transfer pigs. Cultural and microscopic examinations were made on urine sediment.

Case No. XVI

A sample of blood was drawn aseptically from cow No. 373 during November. Urine specimens were collected once during November and four times in December, 1957. Guinea pig No. 980C

was inoculated with 4 cubic centimeters of whole blood, and routine serial transfer was not undertaken. Guinea pigs Nos. 981C, 983C, 986C, 989C, and 990C were inoculated with 0.5 to 1 cubic centimeter of urine sediment. A serial transfer of blood was made four days post-inoculation from each of these (except No. 981C) into guinea pigs Nos. 984C, 988C, 1117, and 991C, respectively. Blood cultures were also made from all of the above guinea pigs. A microscopic and cultural examination was made from the urine sediment.

Case No. XVII

Samples of blood were obtained from cow No. 185B twice in November and once in December, 1957. Urine specimens were collected aseptically twice during November and three times during December, 1957. Guinea pigs Nos. 145Z and 645A were each inoculated with 2 cubic centimeters of direct whole blood. Numbers 735 and 736 received the same blood on the same day. Blood transfers were not made from these guinea pigs. Guinea pigs Nos. 146Z, 738Z, 669A, 910E, and 1008Z were inoculated with 1 cubic centimeter of urine sediment. A serial transfer of blood was made from the latter four pigs into guinea pigs Nos. 750Z, 1088, 927E, and 1023Z. The temperature increased to 106° F. in pig No. 146Z, and blood was withdrawn on the fourth day by cardiac puncture and inoculated (0.5 cubic centimeter) into pig No. 147Z. Blood from pig No. 146Z was also inoculated into four tubes of Stuart's media, using from 3 drops to 0.5 cubic centimeter of

inoculum. Cultures were made from all of the inoculated and transfer guinea pigs. Microscopic and cultural examinations were made of urine specimens.

Case No. XVIII

Samples of blood and urine were obtained from cow No. 266B twice during the month of November and three times in December, 1957. Guinea pigs Nos. 709, 712, and 745 were inoculated with 1 to 5 cubic centimeters of whole blood. Guinea pig No. 710 was inoculated with 1 cubic centimeter of urine sediment, and this pig showed a rise in temperature to 104° F. on the sixth day. Blood was drawn by a cardiac puncture and inoculated in Stuart's media, using 0.1 to 0.5 cubic centimeter. Blood was also inoculated into guinea pig No. 711. Guinea pigs Nos. 713, 746, 872, and 876 were inoculated with urine sediment and Nos. 713 and 746 revealed a thermal reaction of 103.8° F. and 104° F. on the sixth and eighth day. Blood was transferred from Nos. 713 and 746 into guinea pigs Nos. 715 and 747. Blood and culture transfers were also made from pigs Nos. 872 and 876 into pigs Nos, 873 and 891. Cultures were made from the above guinea pigs. Cultural and microscopic examinations were made from urine sediment.

Case No. XIX

Samples of blood and urine were obtained from cow No. 245B four times during the month of December, 1957. Guinea pigs Nos. 623A and 888Z were inoculated with 4 and 5 cubic centimeters of whole blood. Guinea pigs Nos. 628A, 889, 550C, and 901Z were

inoculated with 0.5 to 1 cubic centimeter of urine sediment. A serial flood transfer was made from these pigs into guinea pigs Nos. 629A, 900Z, 555C, and 902Z. Cultures from heart blood were made from each of these pigs. Blood cultures were not made in this case from the cow's blood. Direct cultural and microscopic examinations were made on the urine sediment.

Case No. XX

A sample of blood and urine was obtained from cow No. 148B during the month of December, 1957. Guinea pig No. 1425Z was inoculated with 3 cubic centimeters of blood, and one guinea pig No. 1412 was inoculated with 2 cubic centimeters of urine sediment. Blood (1 cubic centimeter) was transferred from this guinea pig into guinea pig No. 1434. Cultures were made from each of the two guinea pigs. Cultural and microscopic examinations were made on the urine sediment. Whole blood cultures were not made from the original cow blood in this case.

RESULTS

Case No. 1

Two hamsters (Nos. 1 and 2) were inoculated with urine. Number 1 died on the second day due to an accident, and No. 2 failed to develop a thermal reaction. Cultural examination of urine sediment in two tubes of Stuart's media under dark field microscopy did not show the presence of Leptospira during the period of observation. Microscopic examination of urine sediment

and stained preparation did not reveal leptospirae. Blood smears made from the blood of the calf showed changes associated with anemia. The serum sample from this animal gave negative reaction when subjected to the plate agglutination test for leptospirosis. Animal inoculation and cultural and microscopic tests gave negative findings for leptospira.

Case No. II

Two hamsters (Nos. 3 and 4) were inoculated with urine. Number 3 showed a rise in temperature to 104° F. on the seventh day and showed marked dullness, loss of agility, and sticky eyes. This hamster was sacrificed, and 0.5 cubic centimeter of blood was drawn by cardiac puncture and was mixed with 1.5 cubic centimeters of sterile saline. With this dilution, two hamsters (Nos. 5 and 6) were inoculated intraperitoneally; each received 1 cubic centimeter. Necropsy on hamster No. 3 revealed abscesses in the abdominal cavity. Hamsters No. 5 and 6 died on the following night, and necropsy revealed marked hyperemia and areas of scattered hemmorhages. Hamster No. 4 had the same symptoms as No. 3 and died on the seventh night. Necropsy was not performed because of decomposition. Cultural examination of urine-inoculated media under dark field revealed contamination with bacteria, and the cultures were discarded. Microscopic examination of urine sediment, hamsters' tissues on cover slip preparations under dark field, and stained urine smears did not show Leptospira. The hamsters died due to bacterial infection. Histological sections of liver from hamster No. 3 did not show any

significant pathological changes. The kidney showed a dilatation of tubules and numerous areas of round cell infiltration in the cortex portion, and an increase of interstitial connective tissue. Stained sections of liver from hamster No. 5 showed numerous areas of focal necrosis; whereas the kidney sections revealed passive congestion and neutrophilic infiltration, which is perhaps suggestive of bacterial infection.

Case No. III (cow)

Necropsy of this cow revealed the liver to be enlarged and swollen. The subcutaneous tissue exhibited a deep, yellow color. No other gross lesions were seen. Hamsters Nos. 7 and 8, which were inoculated with milk and urine, showed no febrile response and died on the 6th and 15th days, respectively. Tissues from bovine and hamsters were saved for pathological studies. Cultures made from blood, urine, and milk were negative for Leptospira under the dark field during the period of observation. Examination of slides from bovine and hamster tissue under dark field and stained urine smears revealed no evidence of the presence of Leptospira. Histological examination of bovine kidney tissues showed no significant change. Liver sections showed disruption of cord cells and ballooning. Scattered areas of necrosis were also seen in the liver parenchyma. Leptospira could not be seen in silver-stained sections. The histopathological picture of the liver resembled leptospiral infection. Stained sections of the liver from hamster No. 7 showed passive congestion and early cloudy swelling; whereas the kidney sections showed passive congestion and no increase of interstitial connective tissue.

Leptospirae were not demonstrated in the tissues by silver stain.

Case No. IV (calf)

Necropsy revealed no outstanding gross lesions other than a marked icterus condition in the liver. Cultures made from urine sediment did not reveal the presence of Leptospira during the period of observation. Direct microscopic examination of urine sediment, coverslip preparations from bovine kidney under dark field, and stained urine smear, showed no Leptospira. Pathological studies of bovine liver and kidney were of no significance. Tissues from guinea pig No. 17 were unsatisfactory for examination due to decomposition. This pig died during the night following inoculation, perhaps as a result of toxemia from the tissue suspension.

Case No. V (calf)

A diagnosis of leptospirosis was made clinically on this calf. Guinea pigs Nos. 496 and 486, inoculated with blood, did not respond to a febrile reaction. Routine cultures from the heart blood of these guinea pigs did not reveal the presence of Leptospira during the period of observation. A serial transfer of heart blood from these into other guinea pigs, Nos. 421 and 1042, and subsequent blood cultures made, also gave negative results. Serum samples obtained from guinea pigs Nos. 496 and 486, when subjected to the plate agglutination test 20 days post-inoculation, gave a negative reaction. In view of the negative findings in animal inoculation, cultural, and serological tests, perhaps

this was a case of hemoglobinuria of undetermined origin.

Case No. VI (calf)

Guinea pigs Nos. 177 and 188, inoculated with liver and kidney suspension, developed a thermal reaction four days after inoculation. Cultures from the heart blood of these guinea pigs revealed the presence of leptospirae under dark field microscopy after 30 days incubation. This animal, therefore, proved to be a case of leptospirosis by animal inoculation and cultural tests.

Case No. VII

Guinea pigs Nos. 19, 20, 23, 436, 472, and 21, inoculated with blood, did not develop any thermal reaction, and subsequent serial transfer of heart blood from pigs Nos. 436 and 472 into other pigs (Nos. 420 and 473) gave similar negative reactions. Heart blood cultures made from these four pigs gave negative findings for the presence of Leptospira during the routine observation. Guinea pigs Nos. 22 and 24 inoculated with urine, developed a thermal reaction, and cultures made from the heart of these showed organisms other than Leptospira. Guinea pigs Nos. 22, 479, and 472 died four to seven days post-inoculation. Pig No. 22, on necropsy, revealed purulent peritonitis. From pig No. 24 a serial blood transfer was made into pig No. 85, which gave a febrile response. A serial blood transfer was made from pig No. 479 into pig No. 424. Pig No. 466 and subsequent blood transfer from this into pig No. 474 evinced no thermal reaction, and blood cultures made from each of the two did not show Leptospira.

Pig No. 479 died after 20 days due to some intercurrent infections. Cultural examinations of blood and urine sediment did not show Leptospira during the period of observation. Microscopic examination of urine sediment, coverslip preparations from tissues of Pig No. 472, and stained urine smears did not reveal the presence of Leptospira. Routine blood cultures made from each of these pigs did not show Leptospira under dark field examination.

Histological examination of stained sections of the liver of guinea pig No. 472 showed fatty degeneration and cloudy swelling. There was no evidence of any leucocytic infiltration. Lung and kidney tissues showed no significant change. It was not possible to demonstrate Leptospira in silver-stained sections.

Case No. VIII

Guinea pigs Nos. 26, 31, 33, 470, and 425 and hamsters Nos. 27 and 28, inoculated with blood, developed no thermal reaction during observation. Blood transfer from guinea pigs (inoculated with urine) Nos. 453 and 402 into guinea pigs Nos. 102 and 403 gave no thermal response, and heart blood cultures attempted from these four pigs gave negative findings for the presence of Leptospira when examined under dark field. Guinea pigs Nos. 425 and 470 died within one hour, apparently due to shock as a result of the blood inoculation. On the eighth day, pig No. 453 died, and tissues were collected for study. Cultures attempted from direct blood and urine sediment, when examined under dark field microscopy, did not reveal Leptospira during the period of observation. Microscopic examinations of urine sediment, cover slip

preparations of tissues (from pig No. 453) and stained urine smears did not show Leptospira. Pathological studies of stained sections of pig No. 453 did not show any significant change. Silver-stained tissues of the lung, liver, and kidney did not show the presence of Leptospira.

Case No. IX

Blood-inoculated guinea pigs Nos. 35, 37, and 418 did not develop any thermal reaction. Serial transfer from pig No. 418 to guinea pig No. 200 also gave negative thermal reaction. Heart blood cultures made from each of these animals did not show Leptospira under dark field during observation. Guinea pigs Nos. 39 and 409 died within 20 to 30 minutes post-inoculation. This was perhaps due to shock. Guinea pigs Nos. 36, 38, 455, 40, and 410, inoculated with urine sediment and subsequent routine serial blood transfer from the last three pigs into other guinea pigs Nos. 483, 41, and 42, did not evoke a febrile response. Cultures made from heart blood of guinea pigs Nos. 455, 40, and 410 and transfer pigs Nos. 483, 41, and 42 did not reveal the presence of Leptospira when examined under dark field after 5 to 30 days incubation. Examination of cultures from blood and urine sediment did not show Leptospira during the period of observation. Microscopic examinations of urine sediment and cover slip preparations of tissues and stained urine smears from guinea pig No. 410 did not reveal the presence of Leptospira.

Guinea pig No. 410 died nine days post-inoculation, and necropsy revealed no striking lesions except hyperemia of liver

and spleen. Stained sections of liver revealed passive congestion; few of the vessels were filled with red blood cells. A little cellular activity was seen around the triads and also a few areas of round cell infiltration. Spleen sections demonstrated deposition of hemosidern in scattered areas. Kidney sections showed one focal area of round cell infiltration and a few fibroblasts. Silver-stained sections did not reveal the presence of Leptospira.

Case No. X

Guinea pigs Nos. 43, 45, 433, 435, and 440, inoculated with blood, did not show any thermal reaction during the two-week period of observation. Serial transfer of blood from guinea pigs Nos. 433, 435, and 440 into guinea pigs Nos. 301, 117, and 170 revealed no reactions. Heart blood cultures made from these pigs also did not show Leptospira. Urine-inoculated guinea pigs Nos. 44, 46, 434, 437, and 441, and serial transfer of blood from the last three into other guinea pigs, failed to show a febrile reaction. Cultures from the heart blood of pigs Nos. 434, 437, and 441 and transfer pigs Nos. 215, 49, and 522 did not show Leptospira during the period of observation. Cultural examinations of blood and urine sediment and stained smears did not reveal Leptospira. Guinea pig No. 435 (blood-inoculated) died on the eighth day post-inoculation, and on necropsy no gross lesions could be detected. Stained sections of lung, liver, and kidney did not show any significant change. Silver-stained sections of tissues did not reveal the presence of Leptospira in this animal.

Case No. XI

Guinea pigs Nos. 525, 528, 203, 220, and 235, inoculated with blood, failed to develop a thermal reaction. Blood transfers were made from pigs Nos. 203 and 220 into guinea pigs Nos. 204 and 224 and no thermal reaction was given. Heart blood cultures from these pigs did not reveal the presence of Leptospira. Pig No. 235 died due to shock after inoculation with blood. Guinea pigs Nos. 526, 529, 205, 222, and 237 (inoculated with urine) and serial transfers were made from four pigs (excluding No. 529), and the serial transfer guinea pigs, Nos. 527, 109, 232, and 238 failed to elicit a febrile response. Heart blood cultures from these eight guinea pigs failed to show the presence of Leptospira under the dark field. Cultures from urine sediment also did not reveal Leptospira during the period of observation. Microscopic examination of urine sediment and stained urine smears gave no evidence for the presence of Leptospira.

Case No. XII

Guinea pigs inoculated with blood did not develop thermal reactions. Guinea pig No. 429 died the night following inoculation, probably due to shock. Necropsy revealed post mortem decomposition. Guinea pigs Nos. 80 and 110 died immediately following inoculation, due to shock. Blood cultures made from guinea pigs Nos. 1020C and 1021C showed no evidence of Leptospira under dark field examinations. Guinea pigs Nos. 51, 909, 725, 81, and 101, inoculated with urine, developed no thermal reaction. Guinea pigs

Nos. 911, 735, 91, and 102, receiving serial blood transfers, gave no thermal reaction, and heart blood cultures from these pigs did not reveal the presence of Leptospira when examined under dark field. Cultures made from urine sediment did not show the presence of Leptospira during the period of observation. Microscopic examination of urine sediment under dark field microscopy, and urine stained smears did not reveal Leptospira.

Case No. XIII

Guinea pig No. 170Z, inoculated with blood, did not develop a thermal reaction. Guinea pigs inoculated with urine (Nos. 300, 177Z, 665, and 249 did not show a febrile response. A serial transfer of blood was made from the last three guinea pigs into pigs Nos. 810Z, 666, and 250. The guinea pigs receiving the serial transfer also did not show a thermal reaction. The heart blood cultures made from these seven guinea pigs, did not show the presence of Leptospira under the dark field examinations. Microscopic examination of cultures from urine sediment under dark field and stained urine smears gave negative findings for the presence of Leptospira. Stained tissue sections of guinea pig No. 665, that died several weeks after inoculation, were found to be unsatisfactory for examination because of decomposition.

Case No. XIV

Blood-inoculated guinea pigs Nos. 779 and 482 failed to develop a thermal reaction. Leptospira could not be demonstrated in blood cultures made from these two guinea pigs. Pig No. 11Z

died immediately due to shock as a result of inoculation. Guinea pigs inoculated with urine did not show a febrile response. Guinea pigs Nos. 501, 315Z, 137C, and 176, receiving blood transfer from Nos. 500, 313Z, 133C, and 175, did not develop thermal reactions. Leptospira could not be demonstrated by dark field examination in heart blood cultures from these eight guinea pigs. Examination of cultures from urine sediment did not reveal the presence of Leptospira during the period of observation. Microscopic examination of urine sediment and stained urine smear also gave negative findings. Guinea pig No. 500 died four days after inoculation, and necropsy did not show any major gross changes. Stained sections of liver and lung showed no significant change. Sections of the kidney exhibited no change except some areas of albumin cast in the tubules. Leptospira could not be found in sections by silver stain.

Case No. XV

Blood-inoculated guinea pigs Nos. 1219 and 497E did not develop a febrile response, and serial blood transfer from No. 497E into guinea pig No. 599E also gave a negative reaction. Blood cultures made from Nos. 497E and 599E did not reveal the presence of Leptospira on dark field examination. Guinea pigs Nos. 498E, 774, 667, and 569, inoculated with urine, and subsequent heart blood transfer into guinea pigs Nos. 501, 775, 668, and 679 did not develop any thermal reaction during the period of observation. Heart blood cultures made from these guinea pigs also did not reveal the presence of Leptospira when examined under dark field.

One urine-inoculated guinea pig, No. 1220, showed a thermal reaction on the sixth day. Direct examination of heart blood from this pig when subjected to dark field microscopy, revealed organisms other than Leptospira. The blood culture revealed a similar type of organism as was seen in the direct blood of pig No. 1220. Guinea pig No. 1221 did not show a thermal reaction, and the heart blood culture was negative. Cultures made from urine sediment did not reveal the presence of Leptospira during the period of observation. The bacterial cultures isolated above were not classified. Microscopic examination of urine sediment and stained smears did not reveal the presence of Leptospira.

Case No. XVI

Guinea pig No. 980C, inoculated with blood, did not develop a thermal reaction. Guinea pigs Nos. 981C, 983C, 986C, 989C, and 990C, inoculated with urine, and subsequent transfer from the last four into guinea pigs Nos. 984C, 988C, 1117, and 991C, respectively, did not reveal any thermal reaction. Heart blood cultures made from these eight pigs failed to demonstrate Leptospira when examined under the dark field. Cultures from urine sediment did not show the presence of Leptospira during the period of observation. Microscopic examination of urine sediment and stained urine smear did not reveal Leptospira.

Case No. XVII

Blood-inoculated guinea pigs Nos. 145Z and 735 did not develop a thermal reaction. Pigs Nos. 736 and 645A died soon after

blood inoculation, probably due to shock. Cultures from heart blood from pigs Nos. 146Z and 147Z, when examined under dark field, revealed organisms but no Leptospira. The remainder of the guinea pigs inoculated with urine, Nos. 738Z, 669A, 910E, 1008Z, and subsequent transfer of these into guinea pigs Nos. 750, 1088, 927E, and 1023Z did not develop any thermal reaction. Heart blood cultures made from each of the eight guinea pigs did not show Leptospira when subjected to dark field examinations. Cultures made from urine sediment did not show Leptospira under dark field microscopy during the period of observation. Microscopic examination of urine sediment and stained urine smears were also negative for Leptospira.

Case No. XVIII

Guinea pig No. 745, inoculated with blood, died due to shock. Guinea pig No. 710, inoculated with urine, developed a thermal reaction, and a serial blood transfer was made into pig No. 711. Cultures were also made from both of these pigs. Direct examination of heart blood from pig No. 710 revealed organisms other than Leptospira under dark field. The blood cultures from both of these pigs showed similar organisms. Both of the pigs survived. Guinea pig No. 713, also inoculated with urine at a later date, reacted in a similar manner as did the serial transfer pig No. 715. Cultures of the blood from these two pigs showed a bacterial infection as in pig No. 710. Similar results from heart blood cultures were observed in guinea pigs Nos. 746 and 747, which also had been inoculated with urine and serial blood

transfer. Guinea pigs Nos. 872 and 876 and subsequent transfer from these into Nos. 873 and 891 did not develop a thermal reaction. Heart blood cultures from these four pigs were negative for Leptospira. Results of three consecutive urine specimens produced thermal reaction in pigs, but the temperature receded on the fifth or sixth day and all survived except guinea pig No. 746, which died on the ninth day. Cultures made from urine sediment did not show Leptospira under dark field microscopy. Microscopic examination of urine sediment and stained urine smears revealed no Leptospira. Necropsy on pig No. 746 revealed no outstanding lesions, except some slight hyperemia of the internal organs. Stained sections of liver, kidney, and spleen showed no significant change. Silver-stained sections of these tissues did not reveal Leptospira.

Case No. XIX

Guinea pig No. 623A, inoculated with blood, did not develop a thermal reaction. Number 888Z died shortly after inoculation due to shock. Guinea pigs Nos. 628A, 889, 550C, and 901Z, inoculated with urine sediment, and serial transfer from these pigs into pigs Nos. 629A, 900Z, 555C, and 902Z did not develop any thermal reaction. Heart blood cultures made from these guinea pigs did not show Leptospira under dark field examination. Cultures from urine sediment did not reveal the presence of Leptospira during the period of observation. Microscopic examinations of urine sediment and stained urine smears also gave negative

findings. Guinea pig No. 901Z died on the tenth day and on necropsy, no gross lesions could be detected. Stained section of liver showed one area of round-cell infiltration, but no other changes were observed. Kidney and lung sections were also negative for pathological changes. Silver-stained sections of tissue did not reveal the presence of Leptospira.

Case No. XX

Guinea pig No. 1425Z died within one hour after inoculation, probably due to shock. Guinea pig No. 1412Z and routine serial transfer into pig No. 1434Z did not develop a thermal reaction. Heart blood cultures from these pigs did not reveal Leptospira when examined under the dark field. Microscopic and cultural examination of urine sediment also was negative for Leptospira. Necropsy of pig 1425Z revealed emphysema and edema of the lungs, and congestion of the mesenteric vessels. Sections of the lung demonstrated emphysema, ruptured alveoli, and oedema as well as round-cell infiltration in the interstitial tissue.

DISCUSSION

In dealing with field cases, the absence of a conclusive etiological agent suggests that these were perhaps cases of idiopathic hemoglobinuria. Smith (53) reported a serious type of hemoglobinuria of unknown origin, affecting cattle irrespective of age, season, or sex. Cooper (11) stated, in his observations, the incidence of hemoglobinuria associated with Clostridium infection in cattle like Clostridium hemolyticum and perfringens type A,

and also with leptospirosis. He stressed differential diagnosis of all cases of hemoglobinuria. Hemoglobinuria was present in cases Nos. I, II, IV, and V. Bacteriological and pathological examinations failed to show evidence with regard to any disease-producing entities in these cases. Guinea pigs and hamsters inoculated with blood from these cases evoked no febrile response. Guinea pigs inoculated with urine sediment often developed thermal reaction, but in most of the cases, there were some extraneous organisms present in the urine that created the febrile reaction. No Leptospira could be isolated from these cases.

Case No. III presented a definite clinical picture of leptospirosis. The animal had signs of hemoglobinuria and bloody mastitis. The cultural and animal inoculation test failed to detect the presence of Leptospira. The pathology observed in the liver section resembled leptospirosis infection.

The calf in Case No. VI had hemoglobinuria and icterus associated with anemia. This case exhibited almost the same symptoms and lesions as were observed in the previous cases. In animal inoculation tests, the guinea pigs developed a thermal reaction on the fourth day. Cultures made from these pigs showed the presence of Leptospira. In other field cases showing similar lesions and symptoms, an attempt to isolate Leptospira was unsuccessful. It was possible that previous cases were those of idiopathic hemoglobinuria.

Serological investigations on college dairy cattle showed 1.36 per cent animal reactors at 1:160 dilution before abortions (Table 1). A definite rising trend in titer was observed after

Table 1. Summary of the plate agglutination test for leptospirosis on Kansas State dairy cattle conducted during the months of June and October, 1957.

Month	Total animals	Titer at screening		Titer at 1:20	Titer at 1:40	Titer at 1:80	Titer at 1:160	Titer at 1:320	Titer at 1:640	Titer at 1:1280	Titer at 1:2560
		Neg.	Pos.								
June	147	30	97	73	23	8	2	-	-	-	-
Percentage		20.41	65.99	49.66	15.64	5.44	1.36	-	-	-	-
October	99	39	57	57	33	29	27	16	11	5	1
Percentage		39.39	57.58	57.58	33.33	29.29	27.27	16.16	11.11	5.05	1.01

the abortions; 27.27 per cent at 1:160 dilution (Table 1. Stoenner (59) stated that serological reactions indicate significant herd infection before the infection is recognized. Bryans (6) demonstrated the presence of leptospiral agglutinins in the serum of horses with periodic opthalmia and expressed the opinion that diagnosis must be based on a rising agglutinin titer. Cow No. 148B gave a negative plate agglutination test in the month of October and when tested again after vaccination (December 18, 1957), the test was again negative for Leptospira antibodies (Table 2). The nature of consecutive abortion affecting the dairy herd cattle during the month of August, 1957 strongly suggests that abortions were attributable to leptospirosis. Cows were tested for brucellosis after the abortion, and were found to be negative (Table 3).

Table 2. Peak titer in Kansas State College dairy cattle as measured by the plate agglutination test during the month of October, 1957.

Cow No.	Screening	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
185B	+	+	+	+	+	+	±		
455B	+	+	+	+	+	+	±	+	
267B	+	+	+	+	+	+	+	+	
205B	+	+	+	+	+	+	+	+	
373	+	+	+	+	+	+	+	±	±
20B	+	+	+	+	+	+	+		
393*	+	+	+	+	+	+	+		
326B	+	+	+	+	+	+	+		
317C	+	+	+	+	+	+	+	+	+
245**	+	+	+	+	+				
159B	+	+	+	+	+	+			
148B	-	-	-	-					

+ = positive; - = negative; ± = suspect.
* Cow No. 393 was not taken in experiment.
** Cow No. 245 tested on Sept. 16,1957 up to 1:160 dilution only.

Table 3. Results of the plate agglutination test for Brucellosis in cattle that aborted in the Kansas State College dairy herd.

Cow No.	Date of abortion	Result of Brucellosis test*
324	3-13-57	Negative
178A	4-14-57	(sold out)
272B	5-31-57	Not known
120B	8- 2-57	Negative
317C	8-23-57	Was reported negative; 1:100, incomplete on 9-2-57
245B	8-25-57	Negative
257B	8-25-57	Negative
185B	8-25-57	Negative
156B	9- 7-57	Negative before 1:50 on 9-2-57

†

* Conducted by the Agricultural Research Service Laboratory, Topeka, Kansas.

† Eight other cattle were sold or not available that aborted.

All the cattle under experiment were subjected to thorough examination to exclude other possibilities for these abortions. Serum samples were tested from each cow 40 days post-abortion. Thus, there was sufficient time for antibodies to develop. There seems to be a definite fall in post-vaccination titer (Table 4), but still the animals showed positive titer. This presence of antibodies in the serums of these animals after vaccination probably indicates the degree of immunity conferred on the animals as a result of exposure to leptospiral infections and not due to vaccination.

It was observed that guinea pigs and/or hamsters inoculated with blood from serologically positive cattle in the college dairy herd did not develop thermal reactions. The mortality in this

Table 4. Post-vaccination titer in animals under experiment as measured by the plate agglutination test, during the months of November and December, 1957.

Cow No.	Screening	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
317C	+	+	+	+	+	+	+	±
266B	+	+	+	+	±	-	-	-
102B	+	+	+	+	±	-	-	-
185B	+	+	+	+	±	-	-	-
455B	+	+	+	+	±	-	-	-
156B	+	+	+	+	±	-	-	-
159	+	+	+	+	±	-	-	-
20B	+	+	±	-	-	-	-	-
267B	+	+	±	-	-	-	-	-
326C	+	+	±	-	-	-	-	-
373*	+	+	+	+	+	+	+	-
245	+	+	+	+	-	-	-	-
148	-	-	-					

+ = positive
± = suspect
- = negative

group of guinea pigs was due primarily to shock following inoculation. In case of urine-inoculated guinea pigs, the most significant finding is the febrile response evinced in this group. The guinea pigs inoculated with urine sediment from cows Nos. 317, 185, and 266 developed a thermal reaction. This thermal reaction was also observed in the pigs inoculated by blood transfer. This reaction in all probability can be attributed to the organisms that gained entrance in the urine from the urogenital tract of the cow at the time of drawing the urine samples. These organisms did not show any appreciable virulence for guinea pigs. The mortality in this group of pigs occurred due to intercurrent infections.

The characteristic pattern of clinical symptoms and histopathological alterations seen in these experimental guinea pigs

suggests that material obtained from the cattle did not contain any Leptospira.

York, et al. (66) reported in his investigations that cattle exposed to natural attack with Leptospira pomona proved refractory to experimental infection. Calves vaccinated with Leptospira pomona vaccine, when challenged, did not develop infection.

The guinea pigs inoculated with blood and urine that survived in the experiment were all subjected to the plate agglutination test (Table 5) for the detection of antibodies. A negative serological reaction was observed in the serum of all pigs inoculated with blood and urine. Gillespie, et al. (19) stated that exposed cattle vaccinated with L. pomona bacterin several weeks before and subsequently treated with streptomycin, showed a reduction in the shedding of Leptospirae.

The dairy cattle that aborted, exhibited a clinical picture of leptospirosis. It was not possible to isolate or demonstrate Leptospira from the material collected from these cattle by animal inoculation, cultural, microscopic, and pathological techniques. These findings suggest that there were no shedders of leptospirae in this group of cattle under experiment. The animals apparently picked up immunity from the vaccine and the post-vaccination titer was due to previous infection. In all probability, there are some carriers in high-titer animals.

Table 5. Plate agglutination titer in laboratory animals inoculated with urine and blood specimens from suspected cattle infected with leptospirosis in the college herd.

No. of guinea pig or hamster	Date of inoculation	From cow No.	Material inoculated	Date of collection of serum	Screening	1:20	1:40
H. 19	10-23-57	317C	Blood	1-25-58	-	-	-
H. 30	10-23-57	455B	Urine	1-25-58	-	-	-
1220	11-22-57	205B	Urine	1-25-58	-	-	-
36	10-15-57	156B	Urine	1-25-58	-	-	-
35	10-15-57	156B	Blood	1-25-58	-	-	-
44	11-15-57	20B	Urine	1-25-58	-	-	-
433	12-23-57	20B	Blood	1-25-58	-	-	-
525	11-14-57	326B	Blood	1-25-58	-	-	-
529	11-25-57	326B	Urine	1-25-58	-	-	-
738Z	11-29-57	185B	Urine	1-25-58	-	-	-
145Z	11-20-57	185B	Blood	1-25-58	-	-	-
623A	12-17-57	245B	Blood	1-25-58	-	-	-
550C	12-30-57	245B	Urine	1-25-58	-	-	-
981C	11-22-57	373B	Urine	1-25-58	±	-	-
989C	12-30-57	373B	Urine	1-25-58	±	-	-
710	11-13-57	266B	Urine	1-25-58	-	-	-
51	11-25-57	102B	Urine	1-25-58	-	-	-
1020C	12-23-57	102B	Blood	1-25-58	-	-	-
170Z	11-23-57	159B	Blood	1-25-58	-	-	-
177Z	12-20-57	159B	Urine	1-25-58	-	-	-
222	11-27-57	267B	Urine	1-25-58	-	-	-
482C	12-16-57	267B	Blood	1-25-58	-	-	-
418CH*	11-19-57	O.S.L.P.	Blood	1-25-58	+	+	+

* Inoculated with a laboratory culture of L. pomona.
- = Negative.
+ = Positive.
± = Suspect.

CONCLUSIONS

1. Examinations were made on cattle from field cases suffering from a severe type of hemoglobinuria suspected to be infected with leptospirosis.

2. Hemoglobinuria was the predominant symptom in all animals that were submitted for examination.

3. In five of the six field cases, the etiological agent causing the disease could not be recovered.

4. Field Case No. III presented a clinical picture of leptospirosis. Cultural and animal inoculation tests proved inconclusive. The stained liver sections from this animal revealed histopathological alterations associated with leptospirosis.

5. From Field Case No. VI, leptospirae were recovered in culture media after 30 days of incubation. Suspension of bovine kidney and liver tissue produced thermal reaction in the guinea pigs on the fourth day.

6. The nature of abortions in the Kansas State College dairy herd and the presence of high titers (plate agglutination) among cattle was strongly suggestive of leptospirosis. Animals having a high titer were selected for the experiment.

7. Attempts to transmit the disease by inoculation of urine and blood from suspected cases to experimental animals (guinea pigs and hamsters) failed to produce clinical or pathological changes suggestive of the disease.

8. Failure to demonstrate the organisms in the culture media inoculated with urine sediment of suspected cows suggests that leptospiral organisms were not being shed at the time that the urine was collected. However, a few of the guinea pigs showed a thermal reaction but Leptospira organisms could not be recovered in serial transfer or in culture. The thermal reaction that developed in the guinea pigs was apparently due to other microorganisms that were present in the urine. The guinea pigs inoculated with blood showed no thermal reaction. Histological

examinations of tissues from laboratory animals revealed no significant pathology.

9. A plate agglutination test conducted on experimental cattle 45-60 days after vaccination with a bacterin, revealed titers of 1:160 dilution in five of 13 animals. One animal showed a titer of 1:640.

10. Serum samples of guinea pigs inoculated with blood and urine of suspected cows were subjected to the plate agglutination test for the presence of antibodies against L. pomona and were all negative, thus indicating that Leptospira had not been introduced in these experimental animals.

11. On the basis of these findings, the cattle under experiment were not shedding leptospirae in the urine, perhaps as a result of natural immunity or immunity due to vaccination, and hence, the organisms could not be demonstrated by any of the techniques employed in these experiments.

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CARRIER STATE IN LEPTOSPIROSIS-INFECTED ANIMALS FOLLOWING VACCINATION WITH L. POMONA BACTERIN

by

SOHAN LAL ISSAR

G. V. Sc., Bengal Veterinary College,
Calcutta, India, 1942

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This study was undertaken to determine if a carrier state exists in positive animals following vaccination with a bacterin.

Leptospirosis is an infectious disease which affects principally all livestock and domestic animals and is communicable to man from infected animals or animals which may be harboring the infection in a carrier state. There are several species of Leptospira which are responsible for the causation of this malady in humans and animals. The species which affects cattle is Leptospira pomona. The striking symptoms in cattle are decreased or abnormal milk, bloody mastitis, hemoglobinuria, abortions, poor condition, and sometimes death. The L. pomona species is the most important in view of the losses it causes to the livestock industry. The economic loss due to leptospirosis in this country has been established to be over 112 million dollars annually.

The animals used in these experimental trials were from the Kansas State College dairy herd and from field cases suspected for leptospirosis. Animals received from the field were necropsied and material was used for animal inoculation, cultural, microscopic, and pathological investigations. The field cases were used to determine if the methods employed were satisfactory for the isolation of leptospirae. The outstanding symptom in field cases was hemoglobinuria.

Leptospirae were isolated from one of six suspected field cases submitted to the veterinary clinic. It is, therefore, possible that some of the suspected cases were caused by some other etiological agent as was mentioned by other workers. The fact also remains that leptospirae are most difficult to isolate, and these

failures could be due to faulty technique, although this is questionable, as tissue sections did not reveal Leptospira in these cases.

The animals used in this experiment for isolating Leptospira from serologically positive animals which were vaccinated were cattle that had aborted during the month of August, 1957. A serological survey was made in the dairy herd cattle, and those showing a high titer were selected for the experiment. Specimens of blood and urine were obtained from the affected cattle on five different occasions during the months of November and December. The blood and urine samples were inoculated into guinea pigs and/or hamsters, and cultural and microscopic examinations also were utilized on these specimens. The guinea pigs inoculated with blood from the cattle during the course of the experiment did not develop thermal reactions. The guinea pigs inoculated with direct urine sediment revealed a thermal reaction in a few cases, but there were other organisms (bacteria) involved in these reactions. The bacteria were isolated in Stuart's medium from the heart blood of guinea pigs developing a thermal reaction. The microscopic examination of direct urine specimens from cattle on no occasion revealed Leptospirae. Cultures made from these specimens, when subjected to dark field microscopy, did not show the presence of Leptospira. The histopathological examination of hematoxylin-eosin stained tissues from experimental guinea pigs revealed no definite pathology indicative of leptospirosis. Hence, pathological examination gave no evidence as to the presence of leptospirosis in silver-stained sections of the tissues.

Serum samples of guinea pigs that survived inoculations with blood and urine samples were subjected to the plate agglutination test, and no antibodies could be demonstrated by the plate agglutination test in these animals. It is, therefore, possible to infer that the blood and urine specimens collected from the cattle that were serologically positive for Leptospira and vaccinated did not contain leptospirae, as the animals inoculated, cultures, and pathological studies all were negative for this organism.

Serum samples from the cattle under experiment showed a positive serological titer, but there was a definite fall in titer in comparison to the results obtained in October after the abortion. The presence of the post-vaccination titer was perhaps due to previous infection. Previous work has indicated that titers from vaccination are very transitory. Therefore, these titers are possibly a result of previous infection with Leptospira.

It was not possible to isolate leptospirae from aborted cattle three weeks after vaccination with Leptospira bacterin.